Equine Pregnancy: Physical Interactions Between the Uterus and Conceptus

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The equine species have evolved a series of effective, often unique, interrelated, dynamic, physical interactions between the uterus and conceptus. Included are embryo mobility, fixation, and orientation, embryo reduction, formation of endometrial cups, fetal mobility, fetal presentation, uterine horn closures, encasement of the fetal hind limbs by a uterine horn, and special mechanisms of uterine-fetal rotations during parturition. The morphologic and physiologic aspects of these events are described. Author's address: University of Wisconsin, Animal Health and Biomedical Sciences, 1655 Linden Drive, Madison, WI 53706. E-mail: ojg@ahabs.wisc.edu

1. Introduction

This report considers the equine intrauterine conceptus from the time of entry from the oviduct as a blastocyst to expulsion as a foal. A series of events involving physical interactions between the uterus and conceptus will be highlighted, and the associated morphologic and physiologic aspects will be considered. Most of the events were discovered and characterized by transrectal ultrasonic imaging and transcervical endoscopic viewing. Certain aspects of the phenomena have been reviewed for embryos,[1–3] fetuses,[4] and both stages.[5,6]

The term embryo is well engrained and will be used in reference to the entire early conceptus.[5] The terms embryonic vesicle and embryo proper will also be used when needed to emphasize or distinguish between the entire conceptus and the forerunner of the fetus, respectively. The embryo terms will be used only to Day 39 (Day 0=ovulation). From Day 40 to parturition, the terms fetus or fetal stage will be used. The choice of Day 40 as the transition day facilitates categorizing and discussing the phenomena highlighted in this paper. The beginning of umbilical cord formation, completion of replacement of the yolk sac with the allantoic sac, and the beginning of fetal activity (head nods) are on approximately Day 40. Equine theriogenologists and biologists should be deliberate in using the terms embryo and fetus. For example, the terms embryo mobility versus fetal mobility and embryo reduction versus fetal reduction involve distinctly different mechanisms for embryos versus fetuses.

2. The Intrauterine Embryo

A. Origin of Embryonic Placental Layers

Equine theriogenologists should not consider the three germ layers of the embryonic placenta—ectoderm, mesoderm, and endoderm—as esoteric terms used only by embryologists. Transrectal ultrasonography has exposed veterinarians to embryonic placental membranes that formerly received little practical consideration. Knowledge of the layers of the yolk-sac wall and the membranes associated with transition from yolk sac to allantoic sac is required for full interpretation of the ultrasonic

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images of singletons, differentiating singletons from twins, distinguishing between the membranes of impinging uterine cysts and embryonic vesicles, and comprehending today's postulates on the mechanisms of embryo mobility, fixation, orientation, and twin reduction. An embryonic layer that generally is not taught in embryology courses is the capsule, which among farm animals develops only in the equine species. Research on the anatomy of the embryonic placenta is needed because of importance to the equine clinician and scientist. The following discussion of the temporal and spatial interrelationships of the three embryonic layers is based on a small number of embryonic vesicles[5] and should be considered provisional.

The origin of the three germ layers and their incorporation into the yolk-sac wall is diagrammed for Days 9–15 (**Fig. 1**). Diagrams for subsequent embryonic placental development are incorporated into the figures for the various physiologic phenomena.

The embryo becomes a blastocyst by the time or soon after it enters a uterine horn on Day 6.[7] The term blastocyst is used when a central cavity forms, and the inner cell mass is established at one pole, as shown for Day 9.[5] The inner cell mass will form the embryonic disc and eventually will develop into the embryo proper, fetus, and foal. The membrane surrounding the blastocyst cavity is a single layer of

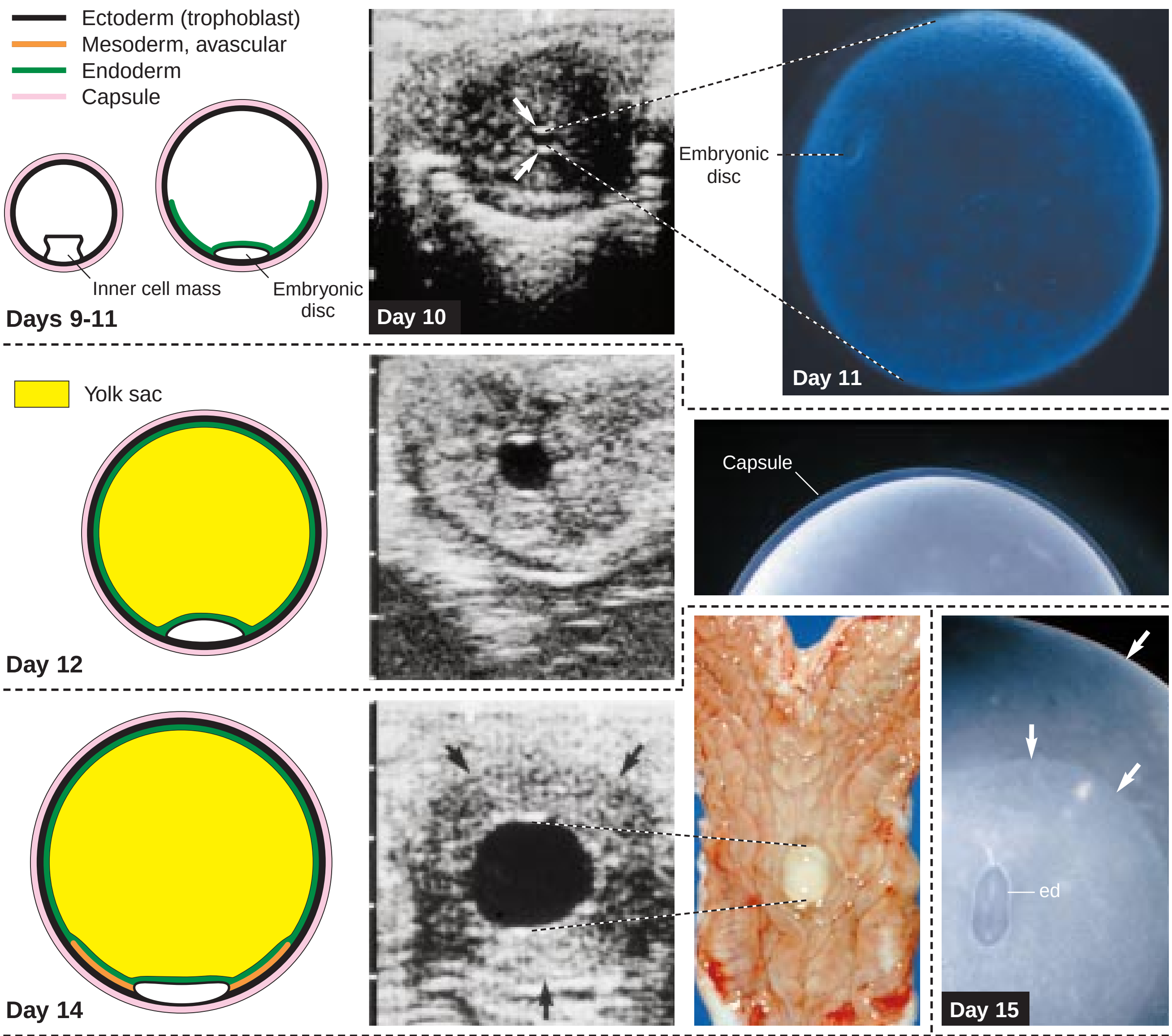

Fig. 1. Diagrams, sonograms, and photographs of the embryo for Days 9–15. The arrows indicate the following: Day-10 sonogram—specular reflections; Day-14 sonogram—periphery of cross-section of the uterine horn; Day-15 specimen—edge of the invading mesoderm (two arrows) and yolk-sac wall (one arrow). The Day-12 specimen shows the capsule after contraction of the blastocyst wall. Note the circular embryonic disc at Day 11 and the elongated disc at Day 15. The head is at the lower end of the Day-15 disc. In these and other sonograms, the graduated scale to the left is in centimeters. ed, embryonic disc.

ectodermal cells called the trophoblast, which continues as the absorptive placental contact with the endometrium throughout pregnancy.

The capsule of an equine blastocyst develops between the trophoblast and zona pellucida.[8] Within a day or so after the blastocyst enters the uterus, the zona pellucida is shed and the capsule becomes the outermost layer. The capsule is a thin (e.g., 3 μm) but tough, mucinous (anti-adhesive[9]) layer of glycoproteins. As the blastocyst expands, the capsule thickens at least until Day 11.[8] The capsule assumes considerable elasticity and resiliency and is a supportive wrapping around a delicate package during embryo mobility (Section 2B), fixation (Section 2C), and orientation (Section 2D). It seems unlikely that these phenomena would have evolved in the absence of the capsule. The capsule disappears by approximately Day 21,[10] suggesting that its role is complete soon after orientation. The popping sensation sometimes experienced by practitioners during elimination of a twin embryo by digital compression[11] is attributable to rupture of the capsule.

Conversion of the single-layered wall of the blastocyst to a two-layered structure occurs upon encirclement of the blastocyst cavity by a single layer of endodermal cells. Although not studied critically, encirclement is completed by approximately Day 12.[5] The resulting primitive placental vesicle can be called a yolk sac, although some authors prefer to continue to call the conceptus a blastocyst until it fixes to the uterus.[10] The endoderm of the yolk sac is continuous with the endoderm of the primitive gut (forerunner of digestive system) of the embryo proper. Therefore, whatever the yolk sac absorbs from the intrauterine environment becomes available to the embryo proper. Beginning growth of the mesoderm from the embryonic disc into the area between the trophoblast and the yolk-sac endoderm is shown in the diagram for Day 14 and by a specimen for Day 15. The invasion of the mesoderm results in a three-layered wall for increasingly greater proportions of the yolk sac, as shown on the diagrams of subsequent figures. The mesoderm differentiates into supportive connective tissue and blood vessels.

Practitioners using an intrarectal 5-MHz ultrasound transducer can view embryos by Days 9–11 when they are 3–5 mm.[6] Only about 5–10% are detectable on Day 9, and then only those that are on the upper end of the Day-9 range; 98% are detectable by Day 11. The vesicles are spherical and produce a black (anechoic) circumscribed image. The spherical shape is maintained with a progressive increase in diameter over Days 9–16. The anechoic area seen by ultrasonography represents the fluid of a blastocyst (Days 9–11) or a yolk sac (Days 12–16). Although invasion by the mesoderm begins by Day 14, the germ layers and capsule are too thin to image individually. Bright white (echoic) spots often are present on the images of the upper and lower surfaces of the blastocyst or yolk-sac parallel to the transducer. These ultrasonically generated specular reflections[12] are an aid in locating the early vesicle. For example, the Day-10 blastocyst in the sonogram may have been missed without the specular reflections. A photograph of a Day-14 conceptus is shown in the uterine body after exposure by an incision along the dorsal uterine midline. The Day-14 vesicle is spherical even though it is freestanding (i.e., not submerged in fluid), indicating that it is a turgid structure. The uterine folds are arranged longitudinally, which may favor embryo mobility.

B. Embryo Mobility and Luteal Maintenance

Uterine ligation studies suggest that the embryo first reaches the uterine body on Day 8[13] and then begins an intrauterine mobility phase that continues until Days 15–17.[14,15] During the embryo-mobility phase, regardless of the side of ovulation, the embryo can be anywhere in the uterine lumen from the tips of either horn to the cervix (**Fig. 2**). When first detected (Days 9-11) the embryo is frequently (60%) found in the uterine body.[14] Thereafter, the frequency of entries into the uterine horns increases as the embryo enters the phase of maximum mobility. The embryo enters every part of the uterine lumen and moves from one horn to another 10 to 20 times per day. Practitioners must be especially aware of the unique embryo mobility characteristic

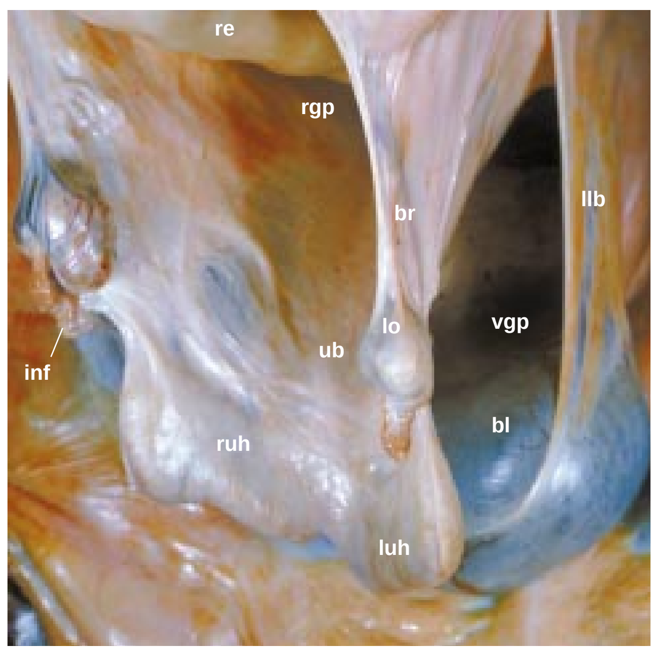

Fig. 2. Frontal-oblique view of suspended reproductive tract after removal of other abdominal viscera.

bl = bladder
br = broad ligament
inf = infundibulum
llb = lateral ligament of bladder
lo = left ovary
luh = left uterine horn
re = rectum
rgp = rectogenital pouch
ruh = right uterine horn
ub = uterine body
vgp = vesicogenital pouch

in this species so that the entire expanse of the uterine lumen is searched during pregnancy diagnosis. Furthermore, the mobility phenomenon is an important aspect of differentiating embryos and cysts and finding and manually eliminating a member of a twin set.

The embryo is subjected to considerable pressure by uterine contractions during embryo mobility. So much so, that the Day 13 or 14 vesicle may undergo periodic compressions (e.g., every 5–14 seconds).[1] During compression, one dimension of the previously spherical vesicle can become twice as great as the other (**Fig. 3**). The compression phenomenon can be seen ultrasonically when the vesicle is viewed in a longitudinal section of the uterus (e.g., uterine body with a linear-array transducer). The widest dimension of the compressed vesicle is in the longitudinal direction of the uterine lumen. Presumably, resiliency and elasticity of the capsule allow uterine-induced distortions of the yolk-sac wall, but provide enough resistance against uterine contractions so that the embryo is moved along the uterine lumen.

Data illustrating embryo mobility in a mare at Day 13[16] and the extent of embryo mobility[14] and uterine contractility[17] on various days are shown (**Fig. 4A,B**). The propulsive force for embryo mobility is uterine contractions as indicated by the following: (1) changes in the extent of uterine contractions, as assessed ultrasonically,[17–20] parallel changes in the extent of mobility (**Fig. 4B**); (2) mobility decreases when uterine contractions are inhibited experimentally[21]; (3) contractions are greater in parts of the uterus exposed to the embryo, based on uterine ligation studies (**Fig. 4C**),[13] indicating that the embryo produces a myometrial stimulant that results in embryo mobility[1]; and (4) the extent of uterine contractility diminishes after the embryo leaves an area.[13] In conclusion, the extensive embryo mobility is attributable to uterine contractions and is favored by the spherical form of the vesicle, the turgidity and anti-adhesive quality of the vesicle resulting from the capsule (Section 2A), and the longitudinal arrangement of the uterine folds (**Fig. 1**).

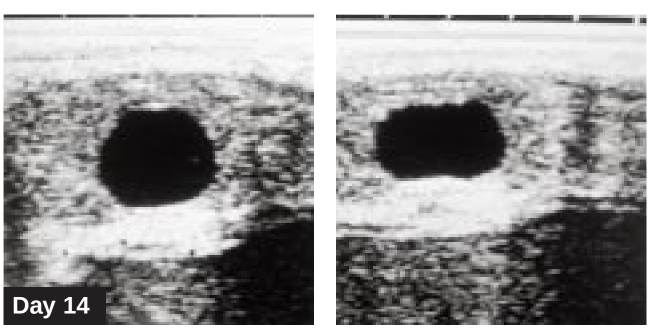

Figure 3. Expansion and contraction of the embryonic vesicle during embryo mobility, as viewed in a longitudinal section of the uterus.

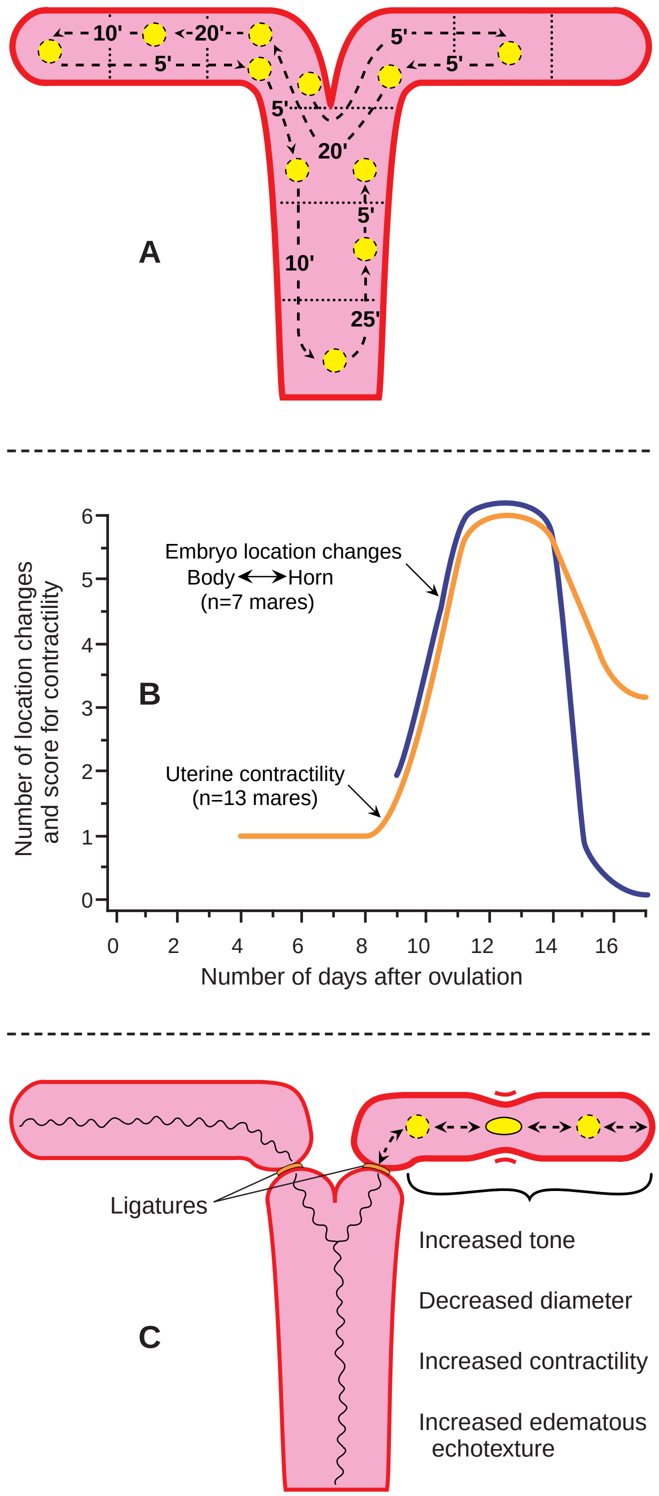

Fig.4. (A) Results of a two-hour mobility trial in a single mare on Day 13. Embryo location was assigned to one of nine arbitrary uterine segments every 5 minutes. The number of minutes required for the embryo to move from one uterine segment to another is shown. (B) Summary of reported data on scores for the extent of uterine contractility and embryo mobility as indicated by the number of location changes between the uterine body and a horn during a two-hour mobility trial. (C) Local effects of the embryo on the uterus as shown by restricting the embryo to one uterine horn.

In the absence of an embryo, the equine uterus produces a potent luteolysin (prostaglandin F2α)[22,23] that travels to the ovary through the systemic circulation, in contrast with the more well-known local or unilateral route in farm ruminants.[24] The indications (reviewed[5,24,25]) of a systemic route for uterine-induced luteolysis in mares versus a local route in cattle are listed (**Table 1**). In farm species, each ovary and the major portion of the uterine tissue on that side is drained by a common vein (uteroovarian vein; **Fig. 5**). In species with a local pathway (e.g., cattle), the ovarian artery is tortuous and is in close apposition to the wall of the uteroovarian vein. In a species with a systemic pathway (horses) the ovarian artery does not contact the uteroovarian vein. The presence or absence of a local utero-ovarian pathway in a given species is attributable to these differences in vascular anatomy.

Table 1. Indicators that the Pathway from Uterus to Ovaries for Uterine-induced Luteolysis is Systemic in Mares and Local in Cows

Item	Mares	Cows
1. Partial hysterectomy	No relationship between side of removal and luteal maintenance [26]	Luteal maintenance only when ipsilateral uterine horn is removed [27]
2. Intrauterine device [24]	Stimulates luteal regression regardless of side relationships	Stimulates luteal regression only if ipsilateral to corpus luteum
3. Route of PGF2α administration for inducing luteolysis [24,28,29]	No differential effect between the IU and IM routes	IU route much more effective than IM route
4. Minimal luteolytic dose of PGF2α [24]	Low (e.g., 5 mg)	High (e.g., 25 mg)
5. Relationship of ovarian artery to uteroovarian vein [25]	Artery and vein not in apposition	Artery tortuous and closely applied to vein

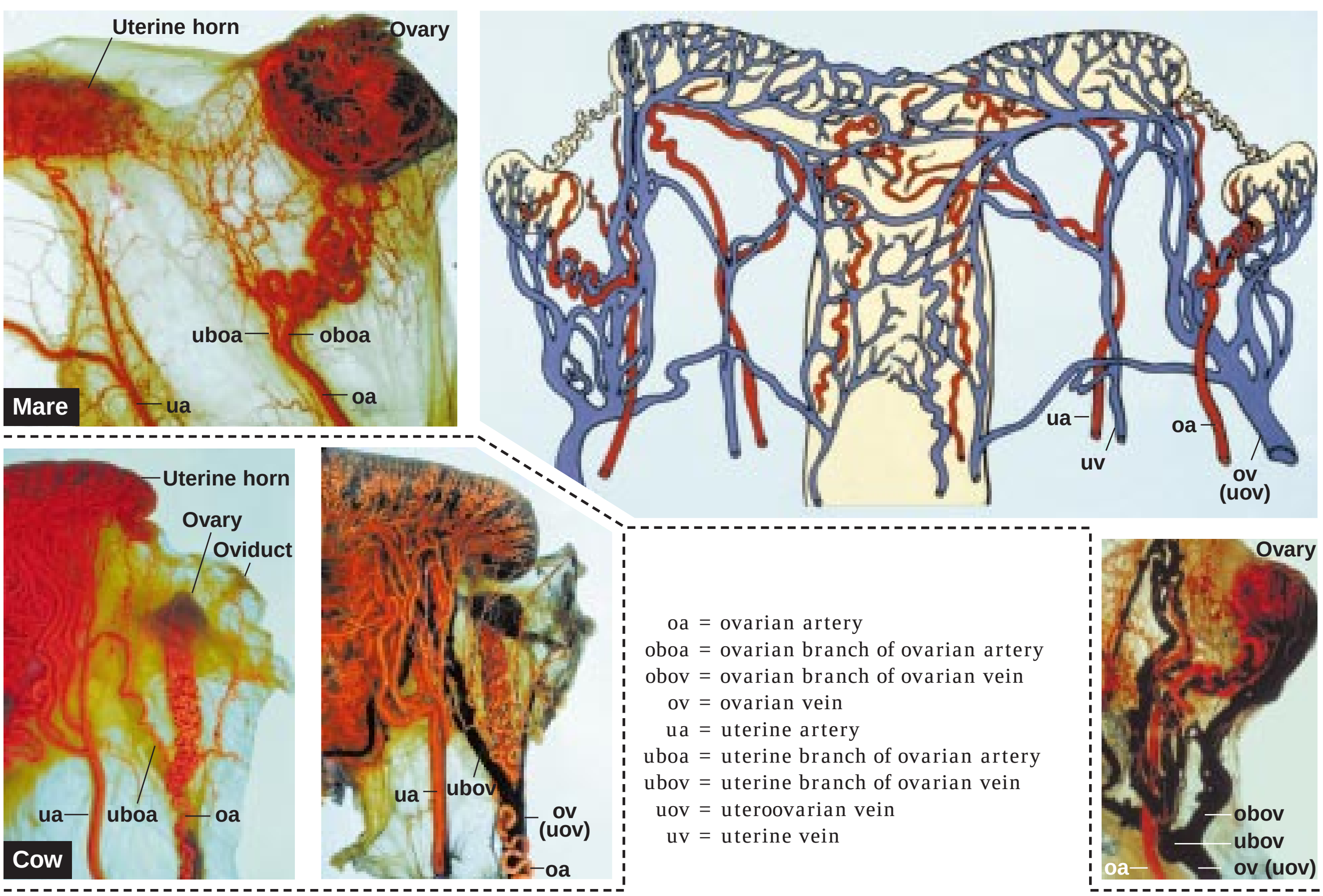

Fig.5. A drawing (upper-right) and photographs of cleared specimens after injection of latex into arteries (red) and veins (blue).

Before the identity of the uterine luteolysin, comparisons of the uteroovarian vascular anatomy of mares (no local pathway) with that of the other farm species provided the earliest suggestion that the pathway between uterus and ovaries was venoarterial (reviewed[24,25]). Experimental vascular anastomoses were used to test the hypothesis of a unilateral venoarterial pathway without assumptions on the identity of the luteolysin. For example, in unilaterally hysterectomized ewes, the uterine vein or ovarian artery from the intact side was anastomosed to the corresponding vessel on the hysterectomized side. The anastomoses allowed luteal regression on the hysterectomized side, whereas luteal maintenance occurred if blood was not shunted from the intact side. Surgical anastomoses between ovarian arteries in ewes with the donor artery originating from various levels of the vascular pedicle delineated the functional area of venoarterial transfer as shown (**Fig. 6**).

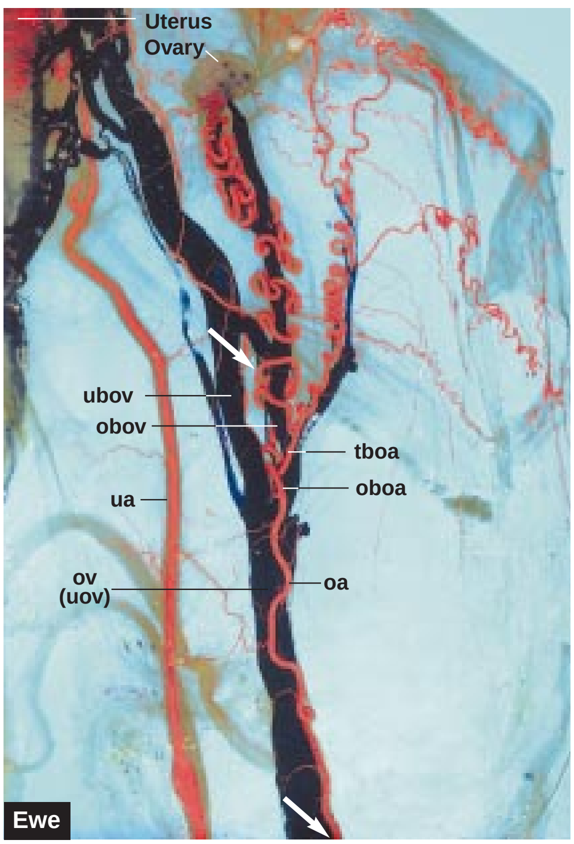

Fig. 6. Specimen of the uteroovarian vascular stem. The two arrows delineate the area of venoarterial transfer of substances between uterine venous blood and ovarian arterial blood. Note the anastomosis (above upper arrow) carrying uterine venous blood into the obov in the convoluted area of the oboa.

oa = ovarian artery
oboa = ovarian branch of ovarian artery
obov = ovarian branch of ovarian vein
ov = ovarian vein
tboa = tubal branch of ovarian artery
ua = uterine artery
ubov = uterine branch of ovarian vein
uov = uteroovarian vein

In the area of apposition between the two vessels, the vessel walls are thin and the connective tissue of the external layers forms a single stratum. Most likely passage of the luteolytic substance through the walls of the vein and artery occurs passively.

When an embryo is present, luteolysis must be blocked (first luteal response to pregnancy[5]) because the corpus luteum through its hormone, progesterone, is vital to embryo development.[30] Evolutionary pressures apparently directed strategies for embryo survival that were compatible with the systemic uteroovarian pathway for uterine-induced luteolysis in mares versus the unilateral pathway in other farm species. The mobility phenomenon in mares allows the embryo to contact all parts of the uterine lining. In this manner, the relatively small spherical embryo is able to block luteolysis despite the relatively large uterus. Restricting the conceptus to a small portion of the uterus by uterine ligation prevents direct contact between the embryo and the remaining portion of the uterus and apparently results in complete or partial luteolysis,[13,31] although more study is needed.[13] It appears that embryo mobility evolved for blocking all of the uterus in mares, whereas expansion of the trophoblast in the uterine horn on the side of the corpus luteum evolved in cattle (**Fig. 7**).

In addition to the reduction in exposure of the corpus luteum to PGF2α, illustrated in the diagrams, there are indications that the conceptus produces a substance that acts as an antiluteolysin at the ovarian level. Experimental anastomoses of uterine veins or ovarian arteries in pregnant sheep and cattle have demonstrated that the venous drainage of the gravid horn contains such a substance. (reviewed[24]) Studies in ewes indicate that prostaglandin E2 (PGE2) is a candidate for the antiluteolysin. In mares, exogenous PGE2 affects uterine contractility and tone during the time of embryo blockage of uterine-induced luteolysis (Section 2C); a role as an antiluteolysin needs to be studied.

C. Fixation

Fixation is defined as the cessation of embryo mobility.[32] Discovery of the phenomena of embryo mobility and fixation is a research milestone because it provided rationale for hypotheses on the following perplexing phenomena: (1) occurrence of fixation almost always in the caudal portion of one of the uterine horns[32]; (2) lack of agreement between side of ovulation and side of fixation[32]; (3) more frequent embryo fixation in postpartum mares in the most involuted horn[33,34]; (4) greater incidence of unilateral than bilateral fixation in mares with twins, especially when the vesicles are of unequal size (Section 6A); and (5) ability of a small conceptus to block the uterine luteolytic mechanism throughout a relatively large uterus (Section 2B).

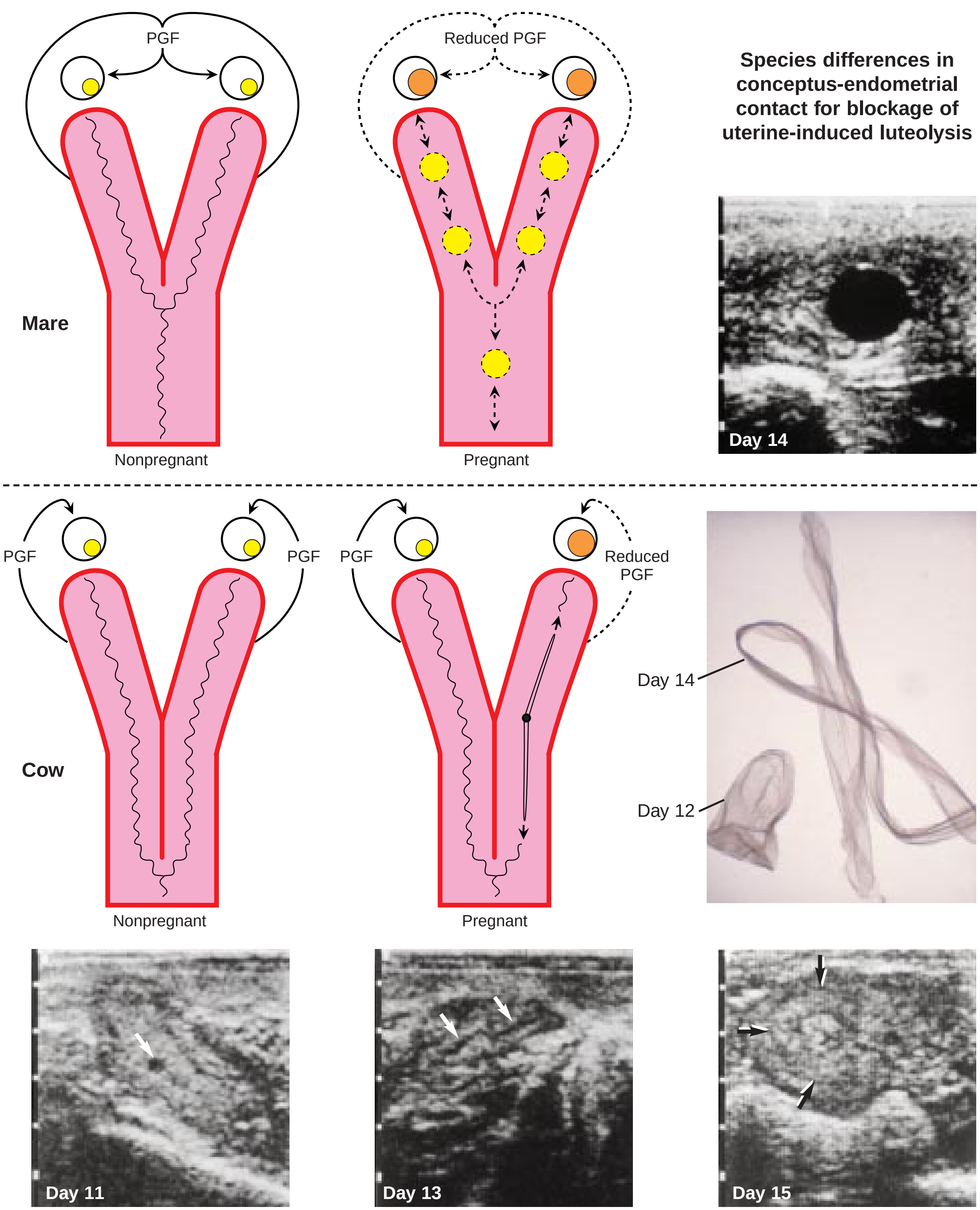

Fig. 7. Embryo-endometrial contact associated with the blockage of uterine-induced luteolysis by the embryo (luteal response to pregnancy). For illustration, the diagrams depict a single embryo and a corpus luteum (regressing=yellow; maintained=orange) in each ovary. The mare has a systemic uteroovarian pathway; the spherical and mobile embryo covers all parts of the uterus and thereby prevents luteal exposure to PGF2α during the critical time (Days 11–15), regardless of luteal location. The cow has a unilateral pathway, and the expanding conceptus during the luteal response to pregnancy covers only the ipsilateral horn. The expansion of the bovine conceptus between days is shown by the sonograms (arrows) and specimens.

On the day of fixation, the vesicle is still spherical as shown by ultrasound studies.[6] At this time, less than half of the yolk-sac wall has developed a third layer (mesoderm), and blood vessels have begun to develop in the mesoderm near the embryo proper (**Fig. 8A**).[5] A cavity, the exocoelom, forms within the mesoderm near the embryo proper, dividing the mesoderm into two layers. Folds of ectoderm and mesoderm begin to pass over the embryo proper and will give rise to the amnion. The membrane consisting of ectoderm and mesoderm is called the chorion and its future is discussed in Section 2E.

Fixation occurs on mean Days 15 in ponies and 16 in horses (**Fig. 8B**).[6] Fixation usually occurs near a flexure in the caudal portion of one of the uterine horns. It has been postulated[32] that fixation occurs at this site, despite continuing uterine contractions,[17–19] because the flexure is the greatest

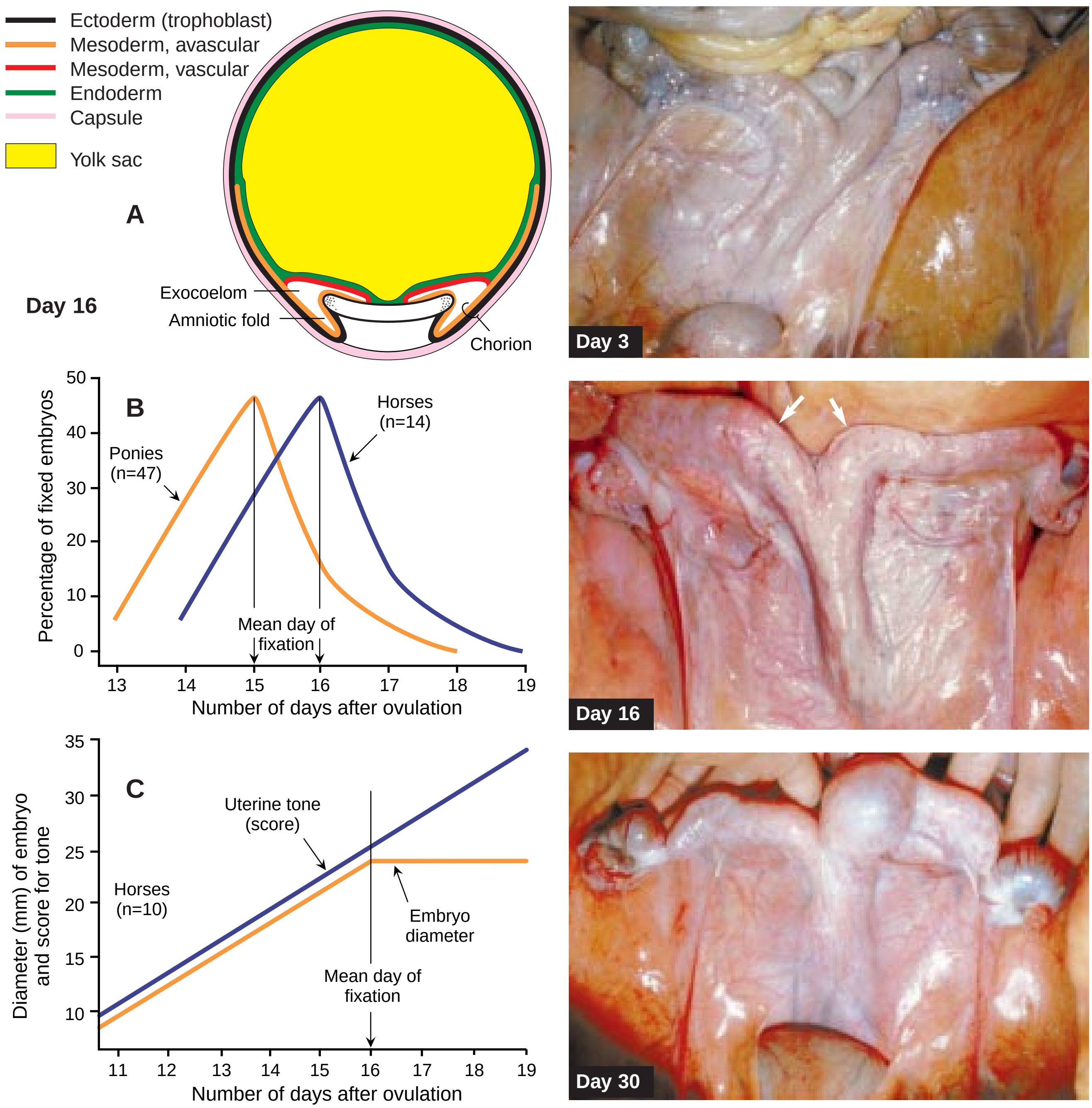

Fig. 8. (A) Diagram of embryo on the day of fixation. (B) Summary of data on frequency of fixation on various days in ponies and horses. (C) Summary of data on temporal association between changing uterine tone and embryo diameter. The photographs depict changes in uterine tone. The increasing uterine tone and embryo diameter are believed to result in the cessation of mobility (fixation) at a flexure (arrows, Day 16) in a caudal uterine horn. Tone increases and confines the embryonal bulge as shown for Day 30.

intraluminal impediment to continued embryo mobility. The photographs show the flexures in the caudal portion of the horns for Day 16 and the corresponding location of the embryonal bulge for Day 30. The uterus rides upon and intermingles with other viscera to a variable extent,[5] and has a T-shape when lying on other viscera.

The equine uterus is flaccid during and immediately after estrus as shown for Day 3, increases in tone until mid-diestrus, decreases in tone until Day 10 or 11, and then, if the mare is pregnant, gradually increases in tone and becomes turgid[20] as shown for Day 16. The increase in uterine tone is associated with a decrease in uterine diameter[34–36] and continues until Days 25–30 as shown for Day 30.[20,37,38] The gradually increasing uterine tone and decreasing uterine diameter together with increasing conceptus diameter[6] apparently combine to result in fixation of the conceptus (**Fig. 8C**).[32] This hypothesis is compatible with the following: (1) earlier fixation and greater uterine tone in young mares than in old mares[39]; (2) higher frequency of fixation in the most involuted horn postpartum[33,34]; (3) the larger the embryo on Day 14, the sooner fixation occurs[18]; (4) fixation when the diameter of the conceptus is similar to the distance between the inner opposite walls of the myometrium of the turgid horns[18]; and (5) fixation a day later in horses than in ponies (**Fig. 8B**).[6] In regard to point 5, the embryo is similar in diameter between the two mare types,[6] but the uterine horns are larger in diameter in horses.[35] Fixation may be aided by a reduction in the slipperiness of the capsule[9] (Section 2B).

The conceptus distributes a tone-stimulating substance during embryo mobility; uterine ligation studies demonstrated that tone was greater in uterine horns exposed to the embryo (**Fig. 4C**).[13] Estradiol may contribute to tone stimulation as indicated by the following: (1) beginning on Day 12, the conceptus produces estrogens in increasing amounts in proportion to its increasing diameter[40]; (2) small doses of exogenous estradiol increased uterine tone in progesterone-primed anestrous mares[37]; (3) exogenous estradiol caused earlier fixation and tended to increase uterine tone[41]; and (4) the confined conceptus locally stimulates estrus-like edematous echotexture of the endometrium.[13] In regard to point 4, moderate edematous changes in the endometrium in early pregnancy do not necessarily indicate impending abortion. Results of a recent study[42] suggest that PGE2, produced by the embryo,[43] plays a role in both stimulating uterine contractions and increasing uterine tone during the embryo-mobility phase. A continuing progesterone source is necessary for embryo mobility and fixation.[30] When luteolysis occurs after fixation, the uterus loses its turgidity, and the embryo leaves the site of fixation. Sometimes the embryonic heart is still beating after the embryo leaves the fixation site; embryonic death, however, is imminent.[44]

To summarize Sections 2A–D (**Fig. 9**), the encapsulated embryo stimulates myometrial contractions and thereby travels throughout the uterus, especially on Days 11–15. The mobile embryo systemically blocks uterine-induced luteolysis and at the same time distributes a substance that gradually stimulates increasing uterine tone. By the time the blockage of luteolysis is complete (mean Day 16), the vesicle has grown and uterine tone has increased (decreased uterine diameter) to the point that fixation occurs in the area of the greatest impediment to mobility (flexure in a caudal horn). This is an exquisite series of events that should pique the curiosity of evolutionists, as well as veterinarians.

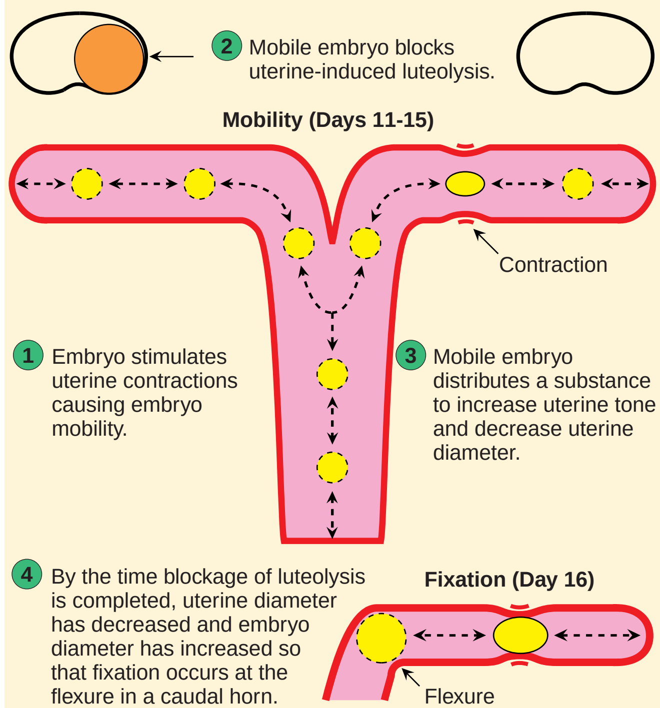

Fig. 9. Summary of events associated with embryo mobility, first luteal response to pregnancy, and embryo fixation.

D. Orientation

Orientation is defined as rotation of the embryonic vesicle so that the embryo proper is on the ventral aspect of the yolk sac.[32] The importance of orientation at this time will become clear in the discussions on the transition between yolk sac and allantoic sac (Section 2E) and formation of the umbilical cord (Section 3A). The preceding diagrams (**Figs. 1,8**) depicted the embryonic disc on the ventral surface of the yolk sac. However, the mobile prefixation vesicle probably rotates, and the embryonic disc can be anywhere on the circumference at this time. In this regard, simulated embryonic vesicles tend to roll when responding to uterine contractions.[1]

The yolk sac is three-layered and vascularized at the embryonic pole and two-layered at the opposite pole (**Fig. 10**).[5] The islands of blood cells that developed in the mesoderm of the three-layered portion have coalesced to form a continuous vascular network. The yolk sac does not contain stored food as in a bird egg, but with vascularization it becomes an efficient purveyor of nutritive material from the uterus to the rapidly developing embryo proper. The amniotic cavity results from a union of the two folds of chorion that pass over the embryo as shown. Closure of the amniotic folds is completed by Day 20,[10] just before loss of the capsule.

Between Days 16 (**Fig. 8**) and 18 (**Fig. 10**), much (>50%) of the yolk-sac wall is still two-layered (no mesoderm)[5] as shown for Day 17 (**Fig. 11**). The mesoderm of the remaining three-layered wall differentiates into connective tissue and a vascular network and is much stronger than the two single-cell layers of the two-layered portion. The difference in strength between the two portions of the yolk-sac wall and the relative expanse of the two portions have been utilized to develop postulates on embryo orientation and embryo reduction in mares with twins (Section 6A). The exposed Day-18 conceptus (**Fig. 10**) does not maintain a spherical shape as it did at Day 14. The sonogram shows that in cross sections of the uterine horn the vesicles tend toward a guitar-pick or irregular shape. The apex of the vesicle is orientated dorsally, and the smooth, rounded base is orientated ventrally. The irregular shapes after fixation are normal and should not be taken as a sign of abnormal development and a harbinger of embryo loss. The shape change is attributable to uterine turgidity and thickening of the dorsal uterine wall, especially on each side of the mesometrial attachment. The vesicle becomes more spherical when an inhibitor of uterine tone is given on Day 19.[45] Because of the uterine turgidity, the vesicle does not enlarge on Days 18–26 when viewed in a cross section of the horn (**Fig. 12**). During this time, a compensatory increase in conceptus length occurs along the longitudinal uterine lumen and accommodates the increasing growth of the conceptus (**Fig. 13**).[6]

Uterine contractions continue after fixation,[17–20] and may play a role in orientating the embryonic

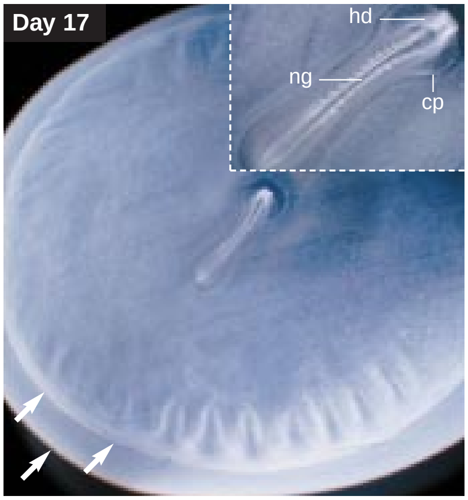

Fig. 11. The peripheries of mesoderm (two arrows) and yolk-sac wall (one arrow) are indicated. The embryo proper (inset) has 11 pairs of somites. cp, cardiac prominence; hd, head; ng, neural groove.

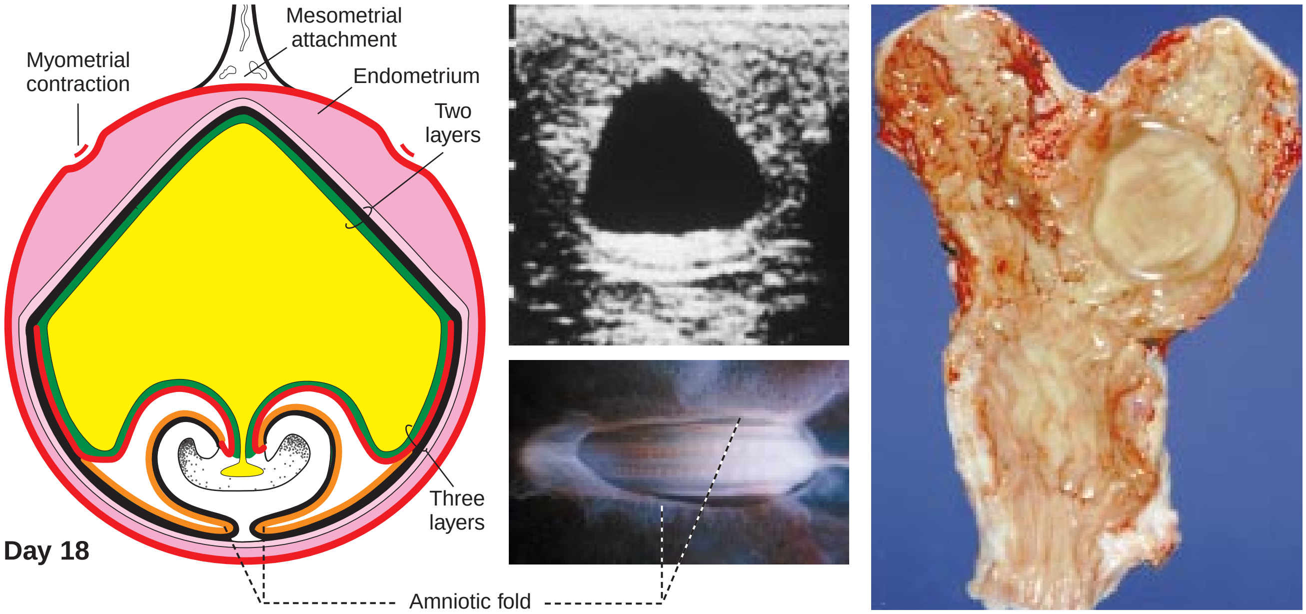

Fig. 10. Diagram, sonogram, and photographs of the embryo on Day 18. The embryo proper is not yet ultrasonically detectable. Thin versus thick portions of the vesicle wall, the continuation of uterine contractions, and disproportional hypertrophy of endometrial folds are believed to result in rotation of the vesicle after fixation. The amniotic folds have not yet closed. For color key, see Fig. 8.

vesicle. Continuous ultrasonic viewing indicates that shape of the fixed embryo is continually altered by uterine contractions (**Fig. 14**).[6] It has been postulated[32] that orientation occurs between the time of fixation and the appearance of the irregular shapes. Three factors are believed[1,32] to interact during orientation: (1) thick (three-layered) and thin (two-layered) portions of the yolk-sac wall; (2) asymmetrical encroachment from thickening of the upper turgid uterine wall on each side of the mesometrial attachment (**Fig. 15**); and (3) the massaging action of uterine contractions. The interaction of these factors results in the thickest portion of the yolk-sac wall (embryonic pole) rotating to a ventral position (antimesometrial). It is not clear, however, whether the localized thickening of the endometrium adjacent to the thin-walled portion of the yolk sac (**Fig. 10**) occurs before, during, or after orientation; the cause of the thickening has not been determined. The orientation hypothesis is compatible with six reported cases of disorientation associated with a flaccid uterus or the presence of twin vesicles at the postulated time of orientation.[1,6] Once fixation and orientation are established, increasing horn turgidity, cranial and caudal to the vesicle (**Fig. 8**), prevents the vesicle from dislodging longitudinally in the uterine lumen. The orientated position, as viewed in cross-section, apparently is aided by adhesiveness and cross-ridging of the endometrial folds.[10] Later, after loss of the capsule on Day 21 (Section 2A), a ridge of trophoblast indents the endometrium and may further anchor the vesicle and help prevent rotation.[10]

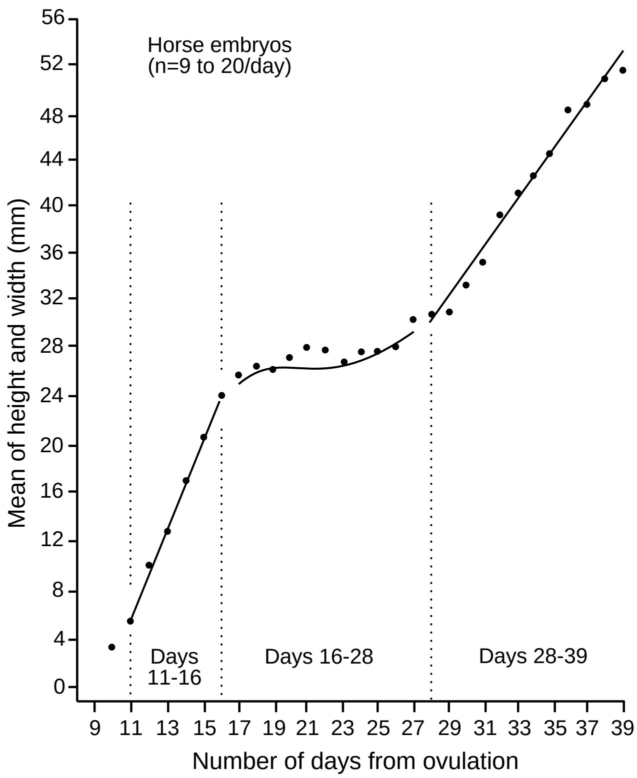

Fig. 12. Cross-sectional growth profile of the embryonic vesicle.

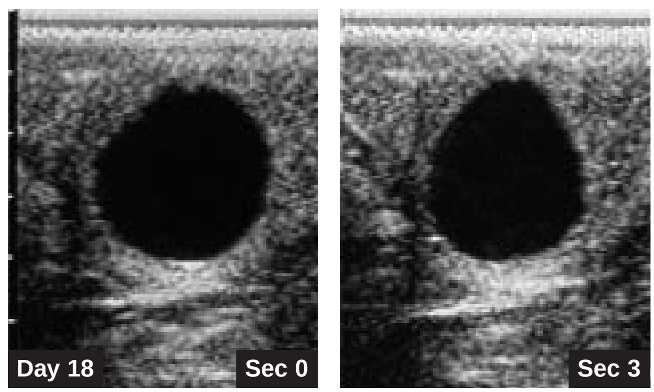

Fig. 14. Example of uterine massage of the vesicle on Day 18. The vesicle changed shape when viewed continuously. The two sonograms were taken at an interval of 3 seconds.

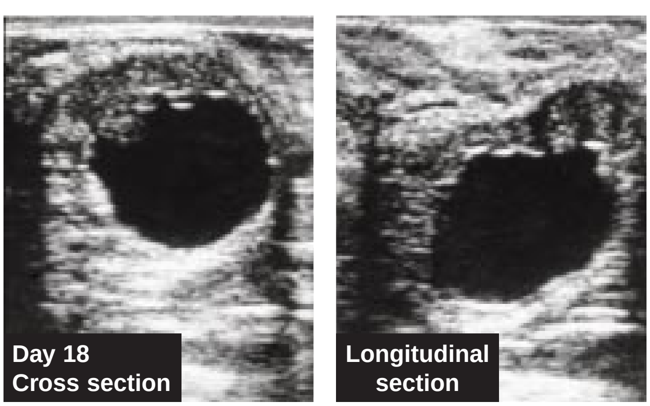

Fig. 13. Cross and longitudinal sections of an embryonic vesicle. The longitudinal section shows that expansion occurs along the uterine lumen and accounts for the plateau in cross-sectional expansion on Days 18–26 that is shown in Fig. 12.

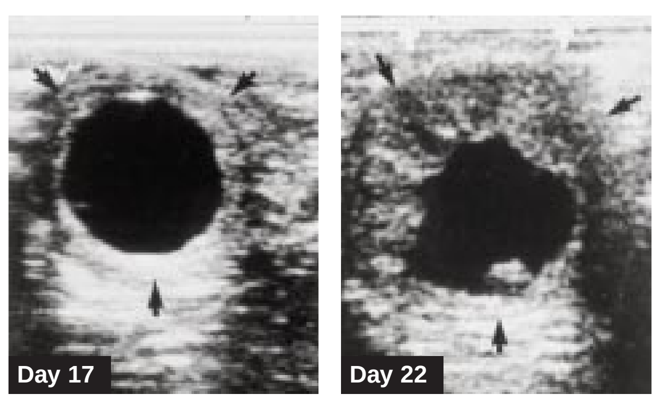

Fig. 15. Cross-sectional views showing the disproportional hypertrophy of the dorsal endometrial folds after Day 17. The outer limits of the uterine wall are delineated by arrows.